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Elevated Levels of Organotins in Lake Geneva: Bivalves as Sentinel Organism

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Organotins, which have widespread applications, are used as biocide agents and in several industrial processes. Tributyltin (TBT) and triphenyltin (TPT) are effective antifouling agents and high concentrations of TBT have been reported in marine (Waldock et al. 1987; Valkirs et al. 1986) and freshwater marinas (Maguire et al. 1986; Zingg 1985). At nanogram per liter levels, TBT may have adverse effects on many aquatic marine organisms (Gibbs and Bryan 1987; Beaumont and Budd 1984; Alzieu and Heral 1984) and is therefore one of the most toxic compounds to aquatic organisms ever introduced deliberately to water (Maguire 1987). As a result of a drastic reduction of the oyster cultures in Arcachon Bay, France, the use of organotin compounds in antifouling paints was prohibited in France in 1982 for vessels less than 25 m in length (Alzieu and Heral 1984). In Switzerland, the use of these paints was not regulated at the time of this study (1988).

In the aquatic environment, TBT is degraded to dibutyltin (DBT), monobutyltin and inorganic tin. The half-life of TBT is up to 4 mon in freshwater, 4 to 5 mon in freshwater sediment (Maguire and Tkacz 1985) and can be as long as 2 yr in deep and anaerobic estuarine sediments (Waldock et al. 1990). As marine bivalves seem to have a limited ability to metabolize TBT (Lee 1986), they have been shown to accumulate it (Laughlin et al. 1986; Wade et al. 1988); but very little has been reported on freshwater clams. Oysters and mussels have been widely used as sentinel organisms to assess the contamination of marine ecosystems by organotin compounds (Wade et al. 1988) or other chemical contaminants (Farrington 1983). In freshwater systems, the bivalve *Dreissena polymorpha* has already been used as bioindicator for heavy metals (Marquenie 1981).

As few data are available concerning organotin contamination of freshwater ecosystems, it seemed to us of great interest to assess for the first time the contamination of Lake Geneva by TBT and its degradation product DBT, as well as TPT, by analysing water, sediment and 2 genera of bivalves. The study was designed to compare the organotin concentrations in marinas within two countries (Switzerland and France) having different legislations. The use of *Dreissena polymorpha* as a sentinel organism for the contamination by organotin compounds has been examined.

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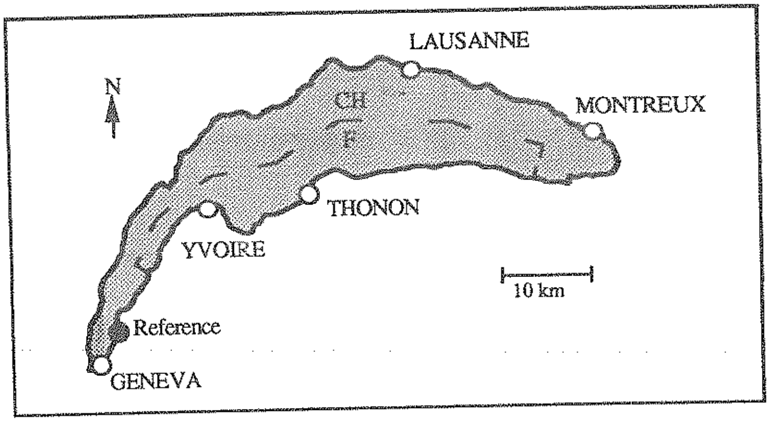

Figure 1. Sampling locations in Lake Geneva (46°27' N, 6°32' E).
Details of sampling locations are available on request.

O = Marina
● = Natural site
CH = Switzerland
F = France

MATERIALS AND METHODS

Water, sediment and mollusc samples were collected from three Swiss and two French marinas (Figure 1). As a reference, a natural site was chosen away from sources of organotin compounds, but presenting similar characteristics to a marina: a sheltered and shallow site. For each of the five marinas, three stations were chosen. In each station, a water sample was taken at 20 cm depth with a glass bottle and at 50 cm above the lake bottom with a "Van Dorn" plexiglass bottle during two campaigns (June and September 1988). For the reference only two stations were sampled. At the same time, two genera of filter-feeding bivalves were collected by divers: *Anodonta cygnaea* and *Dreissena polymorpha* to constitute one composite sample per location and per campaign (2 to 9 organisms per sample (mean 4.8) for *A. cygnaea*; 3 to 66 organisms (mean 35.4) for *D. polymorpha*). Three sediment samples per location were sampled in November 1988 with an aluminium "Ekman" grab of 15 x 15 cm at a depth of 1.5 to 5 m, only the top 2 cm were used for analyses. The water samples were extracted immediately after the sampling while the sediment and the bivalves were deep-frozen at -30°C until extraction.

One liter of lake water was extracted with hexane and 2-hydroxy-2,4,6-cycloheptatrien-1-one (tropolone) according to Zietz and Haag (1986), 15 g of lyophilized sediment with diethyl ether and tropolone applying the method of Müller (1987), and 5 g wet weight of mollusc tissue with diethyl ether/hexane and tropolone according to Sasaki et al. (1988). Only minor changes were made concerning these methods. The extracts were alkylated with a Grignard reagent (MeMgCl) and the concentrated extracts of sediment and mollusc were purified on silica-gel (elution with diethyl ether/hexane) (Müller 1987).

The samples were analysed using a Dani 6800 gas chromatograph equipped with a capillary column (SPB-5, 60 m in length, 0.25 mm i.d., and DB-5, 30 m in length, 0.25 mm i.d.) and a flame photometric detector (double flame Dani 68/72, filter 596

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nm). The detection limit was 115 pg for DBT, 125 pg for TBT and 225 pg for TPT. The limits of quantification were the following: 0.01 µg/L of DBT and TBT in water, 0.002 µg/g dry weight of DBT, TBT and TPT in sediment, and 0.003 µg/g wet weight of DBT and TBT and 0.005 µg/g of TPT in bivalves. The results are expressed as the cation weight (e.g. $(C_4H_9)_3Sn^+$ for TBT); they have not been corrected for recovery (water DBT 51%, TBT 91%, TPT 89%; sediment DBT 91%, TBT 61%, TPT 64%; molluscs DBT 110%, TBT 93%, TPT 56%). Procedural blanks were processed with every set of samples, they were all free from organotin contamination or other interferences.

RESULTS AND DISCUSSION

The results of the first assessment of butyl- and phenyltin compounds in Lake Geneva are reported in Tables 1, 2 and 3 and Figures 2 and 3. The tributyltin concentrations ranged from not detected to 1.08 µg/L in water and from 0.03 to 4.76 µg/g dry weight in sediment (Tables 1 and 2). TBT levels in the Swiss marinas were of the same order of magnitude as those found in Lake Constance (Switzerland; Zingg 1985). The Swiss marinas were up to 20 times more contaminated than the French ones (Figure 2). This difference was more pronounced for the water than for the sediment. It can be explained, in part, by the fact that the use of antifouling paints is regulated in France since 1982. Furthermore, the values might reflect the degree of flushing, as Geneva and Lausanne are the most enclosed marinas with the poorest water exchange. In general, higher aqueous concentrations of dibutyltin and TBT were measured in June than in September. This can be due to the typical spring activities (hosing operations and launching of the yachts, Waldock et al. 1987). The variability concerning the different water samples was high, the differences were important between the stations of the same location (see range in Table 1). Sub-surface and bottom TBT concentrations were not significantly different (data not shown) and have therefore been grouped.

All water and sediment samples contained more TBT than DBT which suggests that degradation takes place assuming that the primary input of butyltin to these areas is from TBT-based antifouling paint. The sediment samples containing the highest levels of TBT were those collected near the crane (station 1 for Geneva and Montreux, station 3 for Lausanne and Thonon, Yvoire does not have a crane; see Table 2), where most of the hosing activities take place and where the boats are launched. The level of organotins in water was lower than in sediment, which confirms that they appear to concentrate there. The natural site showed concentrations below those observed in the marinas, but nevertheless organotin compounds could be detected. This indicates that the contamination extends outside the marinas onto the banks of Lake Geneva, or that other important TBT sources exist (the last point seems improbable). Triphenyltin has been detected in the sediments of each location, it was always present at lower concentrations than TBT.

Bivalves have been frequently used as sentinel organisms for the assessment of organotin contamination in marine ecosystems (Farrington 1983; Wade et al. 1988), therefore two species of freshwater bivalves, *Dreissena polymorpha* (zebra mussel) and *Anodonta cygnaea*, have been analysed during this study. The tissues of these molluscs contained high levels of organotins, especially those collected in Swiss ports (Table 3). In general the pattern of the occurrence of DBT and TBT in bivalves is similar to the pattern of occurrence in water and sediment, although the differences between Swiss and French marinas were less pronounced for the

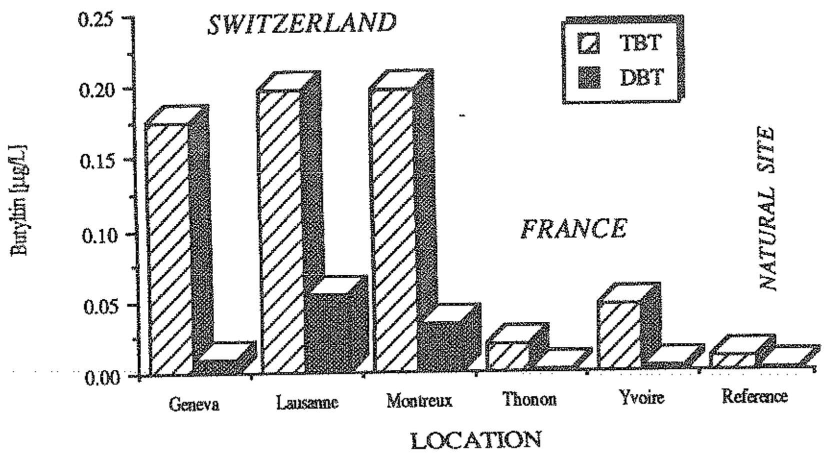

Figure 2. Mean butyltin concentrations in water of Lake Geneva marinas and one natural site.
Each value is a mean of 12 samples (8 samples for the reference) concerning the campaigns of June and September 1988.

Table 1. Concentration of butyltins in water of Lake Geneva marinas and one natural site (reference).

Location	Butyltin concentration [µg/L]			
	June '88		Sept. '88	
	DBT	TBT	DBT	TBT
Geneva (CH)	0.014 (nd-0.083)	0.299 (nd-1.079)	0.005 (nd-0.022)	0.049 (nd-0.087)
Lausanne (CH)	0.072 (nd-0.103)	0.271 (0.077-0.377)	0.037 (nd-0.065)	0.123 (0.079-0.170)
Montreux (CH)	0.061 (nd-0.243)	0.353 (nd-0.695)	0.005 (nd-0.011)	0.041 (nd-0.106)
Thonon (F)	nd	0.015 (nd-0.063)	0.003 (nd-0.008)	0.026 (nd-0.045)
Yvoire (F)	nd	0.047 (nd-0.064)	0.007 (nd-0.010)	0.045 (0.030-0.064)
Reference (CH)	nd	0.013 (nd-0.053)	0.002 (nd-0.008)	0.004 (nd-0.015)

Each value is a mean of 6 samples (3 stations each with one surface and one bottom sample) except for the reference point (2 stations). The range is indicated below in parentheses. TPT has not been detected.
nd = not detected
CH = Switzerland
F = France

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Location		DB'
Geneva (CH)	1	0.1'
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	3	0.2:
Lausanne (CH)	1	0.2'
	2	0.4
	3	1.8
Montreux (CH)	1	0.4
	2	0.0
	3	0.1
Thonon (F)	1	0.0
	2	0.1
	3	0.2
Yvoire (F)	1	0.0
	2	0.1
	3	0.0
Reference (CH)	1	n
	2	0.(

* Each value is a mean
nd = not detected
CH = Switzerland
F = France

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Table 2. Concentration of organotins in sediment of Lake Geneva marinas and one natural site (reference).

Location		Organotin concentration [µg/g dry weight]					
		DBT*	DBT mean	TBT*	TBT mean	TPT*	TPT mean
Geneva (CH)	1	0.175		1.495		0.213	
	2	0.158	0.191	0.686	0.983	0.062	0.137
	3	0.239		0.767		0.137	
Lausanne (CH)	1	0.285		1.086		0.211	
	2	0.465	0.865	1.814	2.555	0.148	0.424
	3	1.846		4.764		0.912	
Montreux (CH)	1	0.448		1.517		0.171	
	2	0.056	0.202	0.176	0.643	0.028	0.081
	3	0.103		0.236		0.043	
Thonon (F)	1	0.047		0.244		0.065	
	2	0.103	0.129	0.470	0.426	0.032	0.037
	3	0.237		0.564		0.014	
Yvoire (F)	1	0.026		0.137		0.017	
	2	0.101	0.061	0.248	0.204	0.010	0.042
	3	0.056		0.227		0.100	
Reference (CH)	1	nd	0.007	0.027	0.034	0.020	0.011
	2	0.014		0.040		0.002	

* Each value is a mean of two analyses of the same sample (top 2 cm).
nd = not detected
CH = Switzerland
F = France

molluscs. The maximal concentration of TBT in the soft parts of *A. cygnaea* (1.68 µg/g wet weight) was about 15 times higher than that measured in *Anodonta sp.* in a Swedish lake (Bjorklund, in Linden 1987). Unfortunately, few other data exist with which to compare our freshwater bivalve results. The amount of TBT in all samples of both species of bivalves was higher than its degradation product, DBT. The TPT concentration in the bivalves was very variable. For some samples, this compound could not be detected because of interferences which completely masked the TPT. In this case, the absence of a TPT peak on the chromatogram does not necessarily certify its absence in the sample.

A. cygnaea accumulated 1.4 to 14.2 times less TBT than *D. polymorpha* (Figure 3), although its lipid content was up to 3.8 times higher (data not shown) and its filtration rate seems to be up to 10 times higher (Mouthon 1982). This difference can be explained, in part, by the biomass (shell length of 1.3 to 3.4 cm for the *D. polymorpha*, and 6.2 to 13.2 cm for the *A. cygnaea* analysed) and by the difference in habitat (*D. polymorpha* lives in the water column while *A. cygnaea* lives in the sediment, filtering the water just above it). TBT concentration was about the same in June and September, but the DBT concentrations increased for *D. polymorpha*, which suggests an increase in degradation.

The highest TBT concentrations observed in the tissues of *D. polymorpha* (9.34 µg/g wet weight in Geneva, June '88) surpass the values given in the literature concerning marine molluscs (Maguire 1987; Wade et al. 1988). Freshwater bivalves, as their marine homologues, are shown to accumulate TBT. *D. polymorpha* present the advantage of having a wide geographical distribution, to